

REMARKS

Claims 75-78, 80, 102, and 105-108 are pending in this application and under examination. No claims are amended in this response.

Applicants acknowledge with gratitude withdrawal of all previous rejections except for obviousness-type double patenting rejections. Applicants also thank the Examiner for considering the information accompanying the Information Disclosure Statement filed on July 27, 2006.

Applicants' response to the double patenting rejections is as follows:

I. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of copending Application No. 10/054,295 (now U.S. Patent 6,921,664). (Office Action paragraph 6.) This rejection is addressed by a terminal disclaimer filed September 29, 2006 (copy enclosed).

II. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of Patent No. 6,475,789. (Office Action paragraph 14). This rejection is addressed by a terminal disclaimer filed September 29, 2006 (copy enclosed).

III. Claims 75-78 and 105-108 were rejected for obviousness-type double patenting over claims of Patent No. 6,261,836. (Office Action paragraph 15.) This rejection is addressed by a terminal disclaimer filed September 29, 2006 (copy enclosed).

IV. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of copending Application No. 10/044,692. (Office Action paragraph 10). A terminal disclaimer with respect to Application No. 10/044,692 is enclosed, overcoming this rejection.

V. Additionally, a terminal disclaimer were filed on September 29, 2006 with respect to U.S. Pat. Nos. 6,927,285 and 6,337,200 (copy enclosed). A terminal disclaimer

with respect to Application No. 10/044,539 (which has not been cited by the Office) is enclosed herewith.

VI. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of copending Application No. 10/877,124. (Office Action paragraph 12.) Copending Application No. 10/877,124 has not yet been examined in the merits, and the present application is expected to issue prior to Application No. 10/877,124. Applicants request that this rejection be withdrawn and applied (if appropriate) during examination of the '124 application.

VII. Claims 75-78 and 105-108 were provisionally rejected for obviousness-type double patenting over claims of copending Application No. 09/721,477. (Office Action paragraph 8.) The pending claims of copending Application No. 09/721,477 stand rejected, and the present application is expected to issue prior to Application No. 09/721,477. Applicants request that this rejection be withdrawn and applied (if appropriate) during examination of the '477 application.

VIII. Claim 75 was rejected for obviousness-type double patenting over claims of Patent No. 6,093,809. (Office Action paragraph 16.) Applicants respectfully traverse this rejection.

U.S. Patent 6,093,809 claims the cDNA sequence of *Euplotes aediculatus* TRT. Accompanying this Response is the article "Reverse transcriptase motifs in the catalytic subunit of telomerase" by Nakamura et al., 1997, Telomerase catalytic subunit homologs from fission yeast and human, *Science* 277:955-9. At page 957, Fig. 2 shows that the hTRT sequence and the *Euplotes* sequence (Ea\_p123) have little similarity. Appendix A of this response is a BLAST alignment of the human and *Euplotes* hTRT amino acid sequences. Using this program, only a 586 amino acid region of the hTRT sequence can be aligned with the *Euplotes* sequence, and sequence identity within this region is only about 21%. The longest exact match is six amino acids in length.

The polynucleotide claimed in the '809 patent does not encode at least 100 contiguous amino acids of the hTRT sequence [claims 105-108 of the present application] or encode a protein that has (i) at least 95% identity to hTRT and (ii) motifs required for telomerase catalytic activity [claims 75-78, 80, and 102 of the present application]. Applicants submit the polynucleotides of the instant application are not obvious variants of the polynucleotides of the '809 patent.

IX. Claims 75-78 and 105-108 were rejected for obviousness-type double patenting over claims of Patent No. 6,767,719. (Office Action paragraph 17.) Applicants respectfully traverse this rejection.

U.S. Patent 6,767,719 claims nucleic acids encoding the protein sequence of mouse TRT (mTRT), and certain homologs thereof. Appendix B of this response is a BLAST alignment of the human TRT and mouse TRT sequences at the amino acid level. The human and mouse TRT full length sequences are only about 62% identical. The longest exact match is 22 consecutive amino acids in length.

The polynucleotide claimed in the '719 patent does not encode at least 100 contiguous amino acids of the hTRT sequence [claims 105 -108 of the present application] or encode a protein that has (i) at least 95% identity to hTRT and (ii) motifs required for telomerase catalytic activity [claims 75 -78, 80, and 102 of the present application]. Applicants submit the polynucleotides of the instant application are not obvious variants of the polynucleotides of the '809 patent.

#### Request for Interview

In the event that the Examiner determines that there are other matters to be addressed before allowance of the application, the undersigned hereby requests an interview by telephone.

Conclusion

All cited patents and applications having been addressed, Applicants respectfully request that the rejections for obviousness-type double patenting be withdrawn, and the application be allowed promptly.

Respectfully submitted,

A handwritten signature in dark ink, appearing to read 'Randolph Ted Apple', with a long horizontal stroke extending to the right.

Randolph Ted Apple  
Registration No. 36,429

November 29, 2006

Enclosures:

Copies of terminal disclaimers filed September 29, 2006.

Terminal disclaimer with respect to copending App. Nos. 10/044,692 and 10/044,539.

Nakamura et al., 1997, Telomerase catalytic subunit homologs from fission yeast and human, *Science* 277:955-9.

Appendix A

Appendix B

## APPENDIX A: SEQUENCE COMPARISON

### Human TRT protein sequence

LOCUS 014746 1132 aa linear PRI 15-JUN-2002  
DEFINITION Telomerase reverse transcriptase (Telomerase catalytic subunit)  
ORGANISM Homo sapiens  
AUTHORS Nakamura,T.M., Morin,G.B., Chapman,K.B., Weinrich,S.L.,  
Andrews,W.H., Lingner,J., Harley,C.B. and Cech,T.R.  
TITLE Telomerase catalytic subunit homologs from fission yeast and human  
JOURNAL Science 277 (5328), 955-959 (1997)

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1 mprapr crav rsllrshyre vlplatfvrr lgpqgwrlvq rgdpaaafra1 vaqclvcvpw
61 darpppaaps frqvscikel varvlqrice rgaknvlafe falldgargg ppeafstsvr
121 sylpntvtda lrgsgawgll lrrvgddvlv hllarcalfv lvapscayqv cgppllyqlga
181 atqarpppha sgprrrlgce rawnhsvrea gvplglpapg arrrggsasr slplkprpr
241 gaapepertz vgggswahpg rtrgspdrgr cvvsparpae eatslegals gtrhshpsvg
301 rqhagppst srpprpwdtp cppvyaetkh flyssgdkeq lrpsflssil rpsltgarrl
361 vetiflgsrp wmpgtprrlp rlpqrywqmr plflellgnh aqcpygvllk thcptraavt
421 paagvcarek pggsvaapee edtdprrlvq llrqhsspwq vygfvracrl rlvpplwgs
481 rhnerrflrn tkkfislghk akslqlcltw kmsvrdcawl rrspgvgcyp aaehtreei
541 lakflhlms yvvvellrsf fyvtettfqk nrlffyrksv wsklqsigir qhlkrvqlre
601 lseaevqrhr earpalltsr lrfipkpdgl rpivnmdivv gartfrrekr aerltsrvka
661 lfsvlryera rrppllgasv lglddihraw rtfvlrvraq dpppelyfvk vdtgaydti
721 pqdrltevia siikpntyc vrryavvqka ahghvrkafk shvstldlq pymrqfvahl
781 qetsplrdav vieqssslne assglfdvfl rfmchhavri rgksyvqcqg ipqgsilstl
841 lcslygdme nklfaglrdd glllrlvddf llvtphltha ktflrtlvrg vpeygcgvnl
901 rktvvnfpve dealggtafv qmpahglfpw cglldtrtl evqsdysya rtsirasltf
961 nrgfkagrm rklfvgvrl kchslfldlq vnsltqvtcn iykllllqay rfhacvqlp
1021 fhqqvwnkpt fflrvlsdta slcysilkak nagmslgakg aagplpseav qwlchqafll
1081 kltrhrtyv plllslrtaq tqslsrklpgt tltaaleaaa palpsdfkti ld

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### Euplotes aediculatus TRT protein sequence

LOCUS AAC47515 1031 aa linear INV 05-MAY-1997  
DEFINITION telomerase subunit p123 [Euplotes aediculatus].  
ORGANISM Euplotes aediculatus  
REFERENCE 1 (residues 1 to 1031)  
AUTHORS Lingner,J., Hughes,T.R., Shevchenko,A., Mann,M., Lundblad,V. and  
Cech,T.R.  
TITLE Reverse transcriptase motifs in the catalytic subunit of telomerase  
JOURNAL Science 276 (5312), 561-567 (1997)  
PUBMED 9110970

CDS 1..1031  
/coded\_by="U95964.1:101..3196"  
/transl\_table=10

#### ORIGIN

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1 mevdvndqad nhgihsalkt ceieikeaktl yswiqkvirc rnqsqshyk dledikifaqt
61 nivatprdyn eedfkviark evfstglmie lidkclvell sssdvdrak lqcfcgqlkg
121 nqlakthllt alstqkqyff qdewnqvram ignelfrhly tkylifqrts egtlvqfcgn
181 nvfdhlkvnd kfdkqkqgga admneprccs tckynvknek dhflnninvp nwnnmksrtr
241 ifycthfnnr nqffkkhefv snknnisamd raqtiftntf rfnrirkklk dkviekiaym
301 lekvdnfnfn yyltkscplp enwrerkqki enlinktree kskyyeelfs yttndkcvdq
361 fineffynil pkdfltgrrn knfgkkykky velnkhelih knlilekint reismqvct
421 sakhfyfydh eniyvlwkl1 rwifedlvvs lircffvyte qqksysktyy yrkniwdvim
481 kmsiadlke tlaevgekev ewkkslgfa pgklrlipkk tfrpimtfn kkivnsdrkt
541 tkltntktll nshlmktlk nrmfkdpfgf avfnyddvmk kyeevfckwk qvgqpk1ffa
601 tmdiekcyds vnreklstfl kttkl1ssdf wimtaqilr knnividskn frkkemkdyf
661 rqkfqkiale ggqyptlfsv leneqndlna kktliveakg rnyfkknll qpvinicqyn
721 yinfngkyf qtkgipqglc vssilssfyf atleesslgf lrdesmnpn pvnllmrlt
781 ddyllittqe nnavlfiek1 invsrengfk fnmklqtsf plpskfaky qmdsveeqni
841 vqdydcwigi sidmktlalm pninlriegi lctlnlnmgt kkasmlwkkk lksflmnnit
901 hyfrktitte dfanktlnk1 fisggykymq cakeykdhfk knlamssmid levskiiysv
961 traffkylvc nikdtifgee hypdflstl khfieifstk kyifnrvcmi lkakeaklks
1021 dqcsliqyd a

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**BLAST COMPARISON — hTRT vs. Euplotes TRT**

Source: <http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi>

Score = 161 bits (407), Expect = 4e-37  
Identities = 132/616 (21%), Positives = 257/616 (41%), Gaps = 43/616 (6%)

Human:	464	FVRACLRRLVPPGLWGSRHNERFLRNTKKFISLGKHAKLSLQELTWKMSVRDCAWLRRS	523	
		F+ ++P R N + F + KK++ L KH + L K++ R+ +W++		
EuplA:	361	FINEFFYNILPKDFTGR-NRKNFQKKVKYVELNKHელიHKNLLLEKINTREISWMQVE	419	
Human:	524	PGVGCVPAAEHRLREEILAKFLHWMMSVYVVELLRSEFFVYTETTFQKNRLFFYRKSVWSK	583	
		+H +L K L W+ VV L+R FFYVTE ++ ++YRK++W		6 amino acids
EuplA:	420	TSAKHFYYFDHE-NIYVLWKLRLWIFEDLVSLIRCFYVTEQKQSYSKTYYYRKNIWDV	478	
Human:	584	LQSIGIRQHLKRVQLRELSEAEVRQHREARPAALLTSRLRFIPKPDGLRPVIMNDYVVGAR	643	
		+ + I LK+ L E+ E EV + +++ +LR IPK RPI+ + +		
EuplA:	479	IMKMSIAD-LKKETLAEVQEKEVEEWKKS-L-GFAPGKLRIPKKTFRPIMTFN----KK	532	
Human:	644	TFRREKRAERLTSRVKALFSLNYERARR---PGLLGASVLGLDDIHRAWRTFVLRVRAQ	700	
		+++ +LT+ K L S L + + G +V DD+ + + FV + + Q		
EuplA:	533	IVNSDRKTTKLTNTKLLNSHMLMLTKLKNRMFKDPFGFAVFNYYDDVMKKYEEFVCKWK-Q	591	
Human:	701	DPPPELYFVKVDVTGAYDTIPQDRLTEVI-----ASIIKPQNTYCVRRYAV	746	
		P+L+F +D+ YD++ +++L+ + A I+K +N +		
EuplA:	592	VGQPKLFFATMDIEKCYDSVNREKLSTFLKTTKLLSSDFWIMTAQILKRKNINVIDSKNF	651	
Human:	747	VQKAAHGHVRKAFKSHVSTLTDLPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLF	806	
		+K + R+ F+ ++ P + + + Q + +++E L		
EuplA:	652	RKKEMKDYFRQKFQK-IALEGGQYPTLFSVLENEQNDLNAKKT LIVEAKQRNYFKKDNLL	710	
Human:	807	DVFLRFMCHHAVRIRGKSYVQCQGIPOQSILSTLLCSLCYGD MENKLFAGIRRD-----	860	
		+ ++ + GK Y Q +GIPOG +S++L S Y +E +R +		
EuplA:	711	QPVINICQYNYINFNGKFYKQTKGIPQGLCVSSILSSFYATLEESSLGLRDESMNPEN	770	
Human:	861	---GLLRLVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLKTVVNFVPEDEALGGT	917	
		LL+RL DD+LL+T +A F+ L+ E G N++K +FP+		
EuplA:	771	PNVNLLMRLTDDYLLITTQENNAVLFIKLINVSRENGFKFNMKKLQTSFPLSPSKFAKY	830	
Human:	918	AFVQMPAHGLF----PWCGLLLDTRTLEVQSDYSSYARTSIRASLTENRGFKAGRNMRRK	973	
		+ + W G+ +D +TL + + + I +L N K +K		
EuplA:	831	GMDSVEEQNIVQDYCDWIGISIDMKTLALMPNINLRIE-GILCTLNLMQTKKASMWLKK	889	
Human:	974	LFGVLRLLKCHSLFLDLQVNSLQTVCTNIYKILLQAYRFHACVLQLPFHQVWKNPTFFL	1033	
		+ + + + + K+ + Y++ C + H + KN		
EuplA:	890	KLKSFLMNNITHYFRKTITTEDFANKTLNKLFISSGGYKYMCAKEYKDHF--KNLAMSS	947	
Human:	1034	RVISDTASLCYSILKA	1049	
		+ + + + YS+ +A		
EuplA:	948	MIDLEVSKIIISVTRA	963	

## APPENDIX B: SEQUENCE COMPARISON

### Human TRT protein sequence

LOCUS 014746 1132 aa linear PRI 15-JUN-2002  
DEFINITION Telomerase reverse transcriptase (Telomerase catalytic subunit)  
ORGANISM Homo sapiens  
AUTHORS Nakamura,T.M., Morin,G.B., Chapman,K.B., Weinrich,S.L.,  
Andrews,W.H., Lingner,J., Harley,C.B. and Cech,T.R.  
TITLE Telomerase catalytic subunit homologs from fission yeast and human  
JOURNAL Science 277 (5328), 955-959 (1997)

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1 mraprcrav rsllrshyre vlplatfvrr lgpqgwrlvq rgdpaafral vaqclvcvpw
61 darpppaaps frqvscikel varvlqrice rgaknvlafe falldgargg ppeafttsvr
121 sylpntvtdda lrgsgawgll lrrvgddvlv hllarcalfv lvapscayqv cgpplyqlga
181 atqarpppha sgprrrlgce rawnhsvrea gvplglpapg arrrggsasr slplkrpr
241 gaapepertz vgggswahpg rtrgpsdrge cvvsparpae eatslegals gtrhshpsvg
301 rqhhaagppst srpprpwdtp cppvyaetkh flyssgdkeq lrpsflsssl rpsltgarrl
361 vetiflgsrp wmpgtprrrlp rlpqrywqmr plfellelgnh aqcpygvlk thcpiraavt
421 paagvcarek pggsvaapee edtdprrrlvq llrqhsspwq vygfvracir rlvppglwgs
481 rhnerrflrn tkkfislghk aklsiqeltw kmsvrdcawt rrspgvgcyp aaehrlreei
541 lakflhwllms vyvvellrsf fyvtettfqn nrlffyrksv wsklqsigir qhlkrvqlre
601 lseaevqrhr earpalltsr lrfipkpdgl rpivnmdivv gartfrrekr aerltsrvka
661 lfsvlneyra rrppllgasv lglddihraw rtfvlrvraq dpppelyfvk vdvtagydti
721 pqdrltevia siikpntyc vrryavvqka ahghvrkafk shvstltdlq pymrqfvahl
781 qetsplrdav viegssslne assglfdvfl rfmchhavri rgksyvqcqg ipqgsilstl
841 lcslygdme nkifagirrd gllrlvddfv llvtphltha ktflrtlvrg vpeygcvnvl
901 rktvvnfpve dealggtafv qmpahglfpw cgllldtrtl evqsdysysa rtsirasltf
961 nrgfkagrnrm rklfvgvlrl kchslfldlq vnslqtvcn iykilillqay rfhacvlqlp
1021 fhqqvwnkpt fflrvlsdta slcysilkak nagmslgakg aagplpseav qwlchqafil
1081 kltrhrvtv pllgslrtaq tqslsrklpgt tltaaleaaan palpsdfkti ld
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### Mouse TRT protein sequence

LOCUS 070372 1122 aa linear ROD 15-JUN-2002  
DEFINITION Telomerase reverse transcriptase (Telomerase catalytic subunit).  
ORGANISM Mus musculus  
AUTHORS Greenberg,R.A., Allsopp,R.C., Chin,L., Morin,G.B. and DePinho,R.A.  
TITLE Expression of mouse telomerase reverse transcriptase during  
development, differentiation and proliferation  
JOURNAL Oncogene 16 (13), 1723-1730 (1998)

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1 mtraprcpav rsllrsryre vwplatfvrr lgpegrrlvq pgdpkiyrtl vaqclvcmhv
61 gsqpppadls fhqvssikel varvvqrice rnernvlafe fellneargg pmaftssvr
121 sylpntviet lrvsgawmll lsrvgddllv yllahcalyl lvppscayqv cgsplyqica
181 ttdiwpvsva syrptrpvgr nftnlrflq iksssrqea kplalpsrgt krhlsitsts
241 vpsakkarcy pvprveegph rqlptpsgk swvpsparsp evptaekdl skgkvsdlsl
301 sgsvckhkp sstslsppr qnafqlrpf ietnflysrg dgqerlnpsf llslqpnlt
361 garrlveif lgsrprtspp lcrthrslrr ywqmrplfq llvnhacqy vllrshcrf
421 rtangqytda lntspplhmd llrlhsspwq vygfiracir kvvsaslwgt rhnerrfkn
481 lkkfislghy qklsiqelmw kmkvedchw rsspdkdrvp aaehrlreri latflfwlmd
541 tyvvqlrsf fyitestfqn nrlffyrksv wsklqsigir qhlervlre lsqeevrhhq
601 dtwampicr lrfipkpngr rpivnmsysm qtralgrkq aqhftqrkt lfsmlnyert
661 khphlmgsav lgmndiyrtw rafvlrvral dqtprmyfvk advtgaydai pggklvevva
721 nmirhsesty cirqyavvrr dsqgqvhsf rrvqvtlsdl qpmgqflkh lqdsdasalr
781 nsvviqsis mnessslfd fflhflrhsv vkigdrctq cggipqgssl stllslcfcg
841 dmenklfaev qrdgllrfv ddfllvtphl dqaktflstl vhgvpaygcm inlqktvvnf
901 pvepgtlgga apyqlpahcl fpwcgllldt qtlevfcdys gyaqtsikts ltfqsvfkag
961 ktmrnklisv lrkchglfl dlqvnslqtv ciniykifil qayrfhacvi qlpfdqrvrk
1021 nltfflgiis sqasccyail kvknpgmtlk asgsfppeaa hwlcyqafil klaahsviyk
1081 clglplrtaq klcrklpea tmtilkaaad palstdfqti ld
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# BLAST COMPARISON — mTRT vs. hTRT

Source: <http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi>

Score = 1340 bits (3468), Expect = 0.0  
Identities = 715/1146 (62%), Positives = 839/1146 (73%), Gaps = 38/1146 (3%)

Mouse: 1 MTRAPRCPAVRSLLRSRYREVWPLATFVRRRLGPEGRRLVQPGDPKIYRTLVAQCLVCMHW 60  
Human: 1 MPRAPRCRAVRSLLRSHYREVLPATFVRRRLGPQGWRVLVQRGDPAAFRALVAQCLVCPW 60

Mouse: 61 GSQPPPADLSFHQVSSLKELVARVVQRLCERNERNVLAFGFELLNEARGGPPMAFTSSVR 120  
Human: 61 DARPAPAAPSFQVSKELVARVLQRLCERGAKNVLAFGFALLDGARGGPPPEAFTTSVR 120

Mouse: 121 SYLPNTVIETLRVSGAWMLLLSRVGGDLLVYLLAHCALYLLVPPSCAYQVCGSPLYQICA 180  
Human: 121 SYLPNTVTDALRGSGAWGLLLRRVGGDVLVHLLARCALFVLVAPSCAYQVCGPPLYQLGA 180

Mouse: 181 TTDIWPVSASRYRTPRPVGRNFTNRLFLQKIKSSSRQEAAPKPLALPSRGTKRHLSTSTS 240  
Human: 181 ATQARPPPHAS-GPRRRLG-----CERAWNHSVREAGVPLGLPAPGARRRGGSASRS 231

Mouse: 241 VPSAKKARCYPVPRVEEGPHRQVLPTPSGKSWVSPAR----SPEVPTAEKDLSSKGKVS 296  
Human: 232 LPLPKRPRRGAPEPERTPVGQGSWAHPGRTRGPSDRGFCVWSPARP-AEEATSLEGALS 290

Mouse: 297 DLSLSG-SVCCKHKPSSSTLLSPPRQNAFQLRP-FIETRHFYLSRGDQGERLNPSFLLSN 354  
Human: 291 GTRHSHPSVGRQHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGD-KEQLRPSFLLSS 349

Mouse: 355 LQPNLTGARRLVEIIFLGSRPRTSGPLCRTHRLSRRYQMRPLFQQLLVNHAECQYVRL 414  
Human: 350 LRPSTLGARRLVETIFLGSRPWMPGTPRRLPRLPQRYQMRPLFLELLGNHAQCPYGVLL 409

Mouse: 415 RSHCRFTA-----NQVTDALNTSPPHMLDLRLHSSPWQVYGFRLACL 459  
Human: 410 KTHCPLRAAVTPAAGVCAREKPGQSVAAPEEEDTPRRLVQLLRQHSSPWQVYGFVRACL 469

Mouse: 460 CKVVSASLWGTRHNERRFFKNLKKFISLGKGLSLQELMWKMKVEDCHWLRSSPGKDRV 519  
Human: 470 RRLVPPGLWGRHNERRFLNRTKKFISLGKHAQLSLQELTWKMSVRDCAWLRSPGVGCV 529

Mouse: 520 PAAEHRLRERILATFLFWMMDTYVVQLLRSFFYITESTFQKNRLLFFYRKSVWSKLQSIGV 579  
Human: 530 PAAEHRLREEILAKFLHWMMSVYVVELLRSFFYVTETTFQKNRLLFFYRKSVWSKLQSIGI 589

Mouse: 580 RQHLEVRVRLRELSQEEVRHHQDTWLAMPICRLRFIPKPNGLRPVNMYSMGTALGRRK 639  
Human: 590 RQHLKRVQLRELSEAEVRQHREARPALTSRLRFIPKPDGLRPVNMVYVVGARTFRREK 649

Mouse: 640 QAQHTQRLKTLFSLNRYERTKHPHLMGSSVLGMNDIYRTWRAFVLRVRLDQTPRMVYFV 699  
Human: 650 RAERLTSRVKALFSLNRYERARRPGLLGASVLGLDDIHRWRTFVLRVRAQDPPPELYFV 709

Mouse: 700 KADVTGAYDAIPQGLKEVVANMIRHSESTYCIQYAVVRRDSQGVHKSFRQVTTLS 759  
Human: 710 KVDVTGAYDTIPQDRLTEVIASIIK-PQNTYCVRRYAVVQKAAHGHVRKAFKSHVSTLTD 768

Mouse: 760 LQPYMGQFLKHLQSDASALRNSVIEQSISSSSSLDFDFLHFLRHSVVKIGDRCYT 819  
Human: 769 LQPYMQFVHLQET--SPLRDVAVIEQSSSLNEASSGLFDVFLRFMCHHAVRIRGKSYV 826

Mouse: 820 QCQGIQPGSSSLTLLCSLCFGDMENKLFAGVQRDGLLLRFVDDFLLVTPHLDQAKTFLST 879  
Human: 827 QCQGIQPGSILSTLLCSLCYGD MENKLFAGIRRDGLLLRLVDDFLLVTPH LTHAKTFLRT 886

22 amino acids



Mouse: 880 LVHGVPEYGCMINLQKTVVNFVPEPGLGGAAPYQLPAHCLFPWCGLLLDTQTLEVFCDY 939  
LV GVPEYGC++NL+KTVVNFVPE LGG A Q+PAH LFPWCGLLLDT+TLEV DY  
Human: 887 LVRGVPEYGCVVNLRKTVVNFVPEDEALGGTAFVQMPAHGLFPWCGLLLDTRTLEVQSDY 946

Mouse: 940 SGYAQTSIKTSLTFQSVFKAGKTMRNKLLSVLRKCHGLFLDLQVNSLQTVCTINIYKIFL 999  
S YA+TSI+ SLTF FKAG+ MR KL VLRLKCH LFLDLQVNSLQTVCTINIYKIFL  
Human: 947 SSYARTSIRASLTFNRGFKAGRNMRRKLFGLRLKCHSLFLDLQVNSLQTVCTINIYKILL 1006

Mouse: 1000 LQAYRFHACVIQLPFDQVRKNLTFFLGISSQASCCYAILKVKNPGMTLKASGS---FP 1056  
LQAYRFHACV+QLPF Q+V KN TFFL +IS AS CY+ILK KN GM+L A G+ P  
Human: 1007 LQAYRFHACVLQLPFHQVWKNPTFFLRVISDTASLCYSILKAKNAGMSLGAKGAAGPLP 1066

Mouse: 1057 PEAHHLWCYQAFLLKLAHLSVIYKCLLGLRRTAQKLLCRKLPEATMTILKAAADPALSTD 1116  
EA WLC+QAFLLKL H V Y LLG LRTAQ L RKLP T+T L+AAA+PAL +D  
Human: 1067 SEAVQWLCHQAFLLKLTRHRVTYVPLLGSRLTAQTQLSRKLPGTTLTALEAAANPALPSD 1126

Mouse: 1117 FQTILD 1122  
F+TILD  
Human: 1127 FKTILD 1132

mine. A single dose of clozapine increases dopamine release in the primate prefrontal cortex, and long-term administration increases basal extracellular dopamine concentration in the prefrontal cortex (21). Although this may not be the only mechanism by which clozapine elicits its effects on PCP-induced cognitive dysfunction, this activation of the dopamine system of the prefrontal cortex may contribute to the ability of clozapine to ameliorate the impairments in our model and, perhaps, in schizophrenia.

Our data show that repeated administration of PCP inhibits basal and stimulated dopaminergic function in the prefrontal cortex of the monkey brain. The deficiency of dopamine in the prefrontal cortex that is induced by repeated administration of PCP is associated with a long-lasting cognitive deficit, which is ameliorated by the atypical therapeutic drug clozapine. These effects are observed long after PCP administration is stopped and thus cannot be attributed to direct effects of the drug. This primate model of dopamine dysfunction in the cortex may provide a paradigm for investigating the pathophysiology underlying neuropsychiatric disorders associated with a primary cognitive dysfunction in the cortex and a dopaminergic deficit in the prefrontal cortex, as is hypothesized in schizophrenia (22). It also may provide a means for evaluating therapeutic agents that are selectively targeted toward alleviating cortical dopamine hypofunction.

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6 December 1996; accepted 17 June 1997

## Telomerase Catalytic Subunit Homologs from Fission Yeast and Human

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Catalytic protein subunits of telomerase from the ciliate *Euplotes aediculatus* and the yeast *Saccharomyces cerevisiae* contain reverse transcriptase motifs. Here the homologous genes from the fission yeast *Schizosaccharomyces pombe* and human are identified. Disruption of the *S. pombe* gene resulted in telomere shortening and senescence, and expression of mRNA from the human gene correlated with telomerase activity in cell lines. Sequence comparisons placed the telomerase proteins in the reverse transcriptase family but revealed hallmarks that distinguish them from retroviral and retrotransposon relatives. Thus, the proposed telomerase catalytic subunits are phylogenetically conserved and represent a deep branch in the evolution of reverse transcriptases.

Telomerase is a ribonucleoprotein enzyme responsible in most eukaryotes for the complete replication of chromosome ends, or telomeres (1). Its RNA subunit provides the template for addition of short sequence repeats [typically 6 to 26 nucleotides (nts) to the chromosome 3' end (2)]. In ciliated protozoa and yeast, telomerase is regulated and the average telomere length is maintained (3). In most human somatic cells, however, telomerase activity cannot be detected and telomeres shorten with successive cell divisions (4). Telomerase activity

reappears in immortalized cell lines and in about 85% of human tumors, which has led to studies of the usefulness of telomerase for cancer diagnostics and therapeutics (5, 6).

Telomerase RNA subunits have been identified and analyzed in ciliates, yeast, and mammals (2, 7), but the protein subunits have been elusive. In *Tetrahymena*, two telomerase-associated proteins (p80, p95) have been described (8), and p80 homologs have been found in humans and rodents (9); the presence of catalytic active site residues in these proteins has not been

established. Purification of telomerase from the ciliate *Euplotes aediculatus* yielded two proteins, p123 and p43 (10), that appear unrelated to p80 and p95 (11): p123 contains reverse transcriptase (RT) motifs and is homologous to yeast Est2 (Ever shorter telomeres) protein (11), which is essential for telomere maintenance in vivo (12). The RT motifs of Est2p are essential for telomeric DNA synthesis in vivo and in vitro (11, 13), supporting the conclusion that Est2p and p123 are the catalytic subunits of telomerase. The question remained whether there are two telomerases in biology, one based on p80- and p95-like proteins and one on p123/Est2p-like proteins.

To determine if Est2p/p123 is conserved among eukaryotes, we searched for homologs in the fission yeast *S. pombe* and humans. Polymerase chain reaction (PCR) amplification of *S. pombe* DNA was carried out with degenerate-sequence primers designed from the *Euplotes* p123 RT motifs B' and C. Of the four prominent products generated, the ~120-base pair (bp) band encoded a peptide sequence homologous to p123 and Est2p. Using this PCR product as a probe for colony hybridization, we identified two overlapping clones from a genomic library and three from a cDNA library (14). None of the three cDNA clones was full length, so we used RT-PCR to obtain the NH<sub>2</sub>-terminal sequences (15). This putative telomerase reverse transcriptase gene, *trt1*<sup>+</sup>, encoded a basic protein with a predicted molecular mass of 116 kilodaltons (kD) (Fig. 1A). The sequence similarity to p123 and Est2p was especially high in the seven RT motifs (Table 1) and in motif T (Telomerase-specific) (Fig. 2). Fifteen introns, ranging from 36 to 71 bp, interrupted the coding sequence. All had consensus splice and branch site sequences (16).

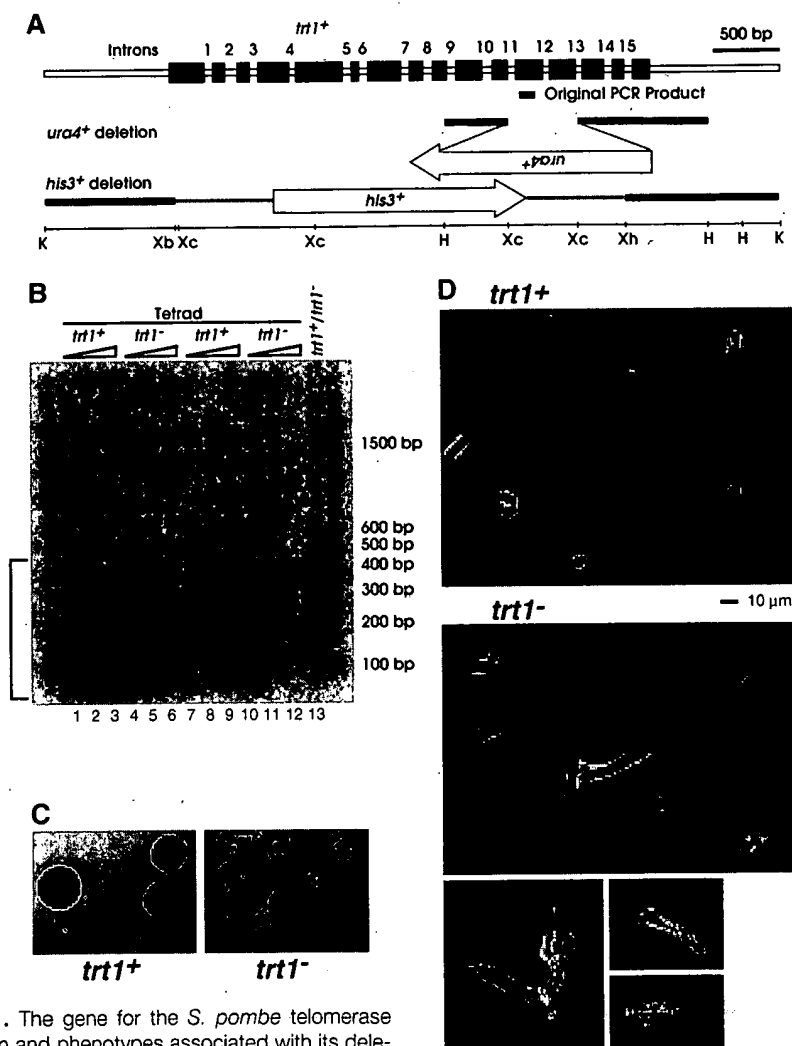
If *trt1*<sup>+</sup> encodes the telomerase catalytic subunit in *S. pombe*, deletion of the gene would be expected to result in telomere shortening and perhaps cellular senescence as seen with the *est2* mutants in *S. cerevisiae* (11, 13). To test this, we created two deletion constructs (Fig. 1A), one removing motifs B' through E in the RT domain, and the second deleting 99% of the open reading frame (ORF). Haploid cells grown from both types of spores showed progressive telomere shortening to the point where hybridization to telomeric repeats became al-

most undetectable (Fig. 1B). Senescence was indicated by (i) reduced ability of the cells to grow on agar, typically by the fourth streak-out after germination; (ii) the appearance of colonies with increasingly ragged edges (Fig. 1C); and (iii) the increasing fraction of elongated cells (Fig. 1D). When individual enlarged cells were separated on the dissecting microscope, the majority underwent no further division. The same *trt1*<sup>-</sup> cell population always contained normal-size cells that continued to divide but frequently produced nondividing prog-

eny. The telomerase-negative survivors may use a recombinational mode of telomere maintenance as documented in budding yeast strains with deletions of telomere-replication genes (12, 17).

A candidate human p123/Est2p/Trt1p homolog was identified by a BLAST search of the EST (expressed sequence tag) database (GenBank AA281296). This EST was the top-ranked match in sequence searches with *Euplotes* p123 ( $P = 3.3 \times 10^{-6}$ ) and *S. pombe* Trt1p ( $P = 9.7 \times 10^{-7}$ ). The human EST was not found in searches with yeast

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**Fig. 1.** The gene for the *S. pombe* telomerase protein and phenotypes associated with its deletion. (A) The *trt1*<sup>+</sup> locus, the location of the ~120 bp PCR product that led to its identification, and the regions replaced by *ura4*<sup>+</sup> or *his3*<sup>+</sup> genes in the *trt1*<sup>-</sup> mutants (K, Kpn I; Xb, Xba I; H, Hind III; Xc, Xca I; Xh, Xho I). (B) Telomere shortening phenotype of *trt1*<sup>-</sup> mutants. A *trt1*<sup>+</sup>/*trt1*<sup>-</sup> diploid (28) was sporulated and the resulting tetrads were dissected and germinated on a YES (Yeast Extract medium Supplemented with amino acids) plate (29). Colonies derived from each spore were grown at 32°C for 3 days, and streaked successively to fresh YES plates every 3 days. A colony from each round was placed in 6 ml of YES liquid culture at 32°C and grown to stationary phase, and genomic DNA was prepared. After digestion with Apa I, DNA was subjected to electrophoresis on a 2.3% agarose gel, stained with ethidium bromide to confirm approximately equal loading in each lane, transferred to a nylon membrane, and hybridized to a telomeric DNA probe. The Apa I site is located 30 to 40 bp away from telomeric repeat sequences in chromosomes I and II. (C) Colony morphology of *trt1*<sup>+</sup> and *trt1*<sup>-</sup> cells. Cells plated on MM [Minimal Medium (29) with glutamic acid substituted for NH<sub>4</sub>Cl] were grown for 2 days at 32°C prior to photography. (D) Micrographs of *trt1*<sup>+</sup> and *trt1*<sup>-</sup> cells grown as in (C).

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Est2p, but subsequent pairwise comparison of these sequences showed a convincing match. Sequencing of the rest of the cDNA clone containing the EST revealed all eight TRT (Telomerase Reverse Transcriptase) motifs, but not in a single ORF (18). We used the sequence information from this incomplete cDNA clone to isolate an extended cDNA clone from a library of 293 cells, an adenovirus E1-transfected human embryonic kidney cell line (19). This cDNA clone (pGRN121) had a 182-bp insert relative to the EST clone, which increased the spacing between motifs A and B' (18) and put all seven RT motifs and the telomerase-specific motif T motifs in a single contiguous ORF (Fig. 2).

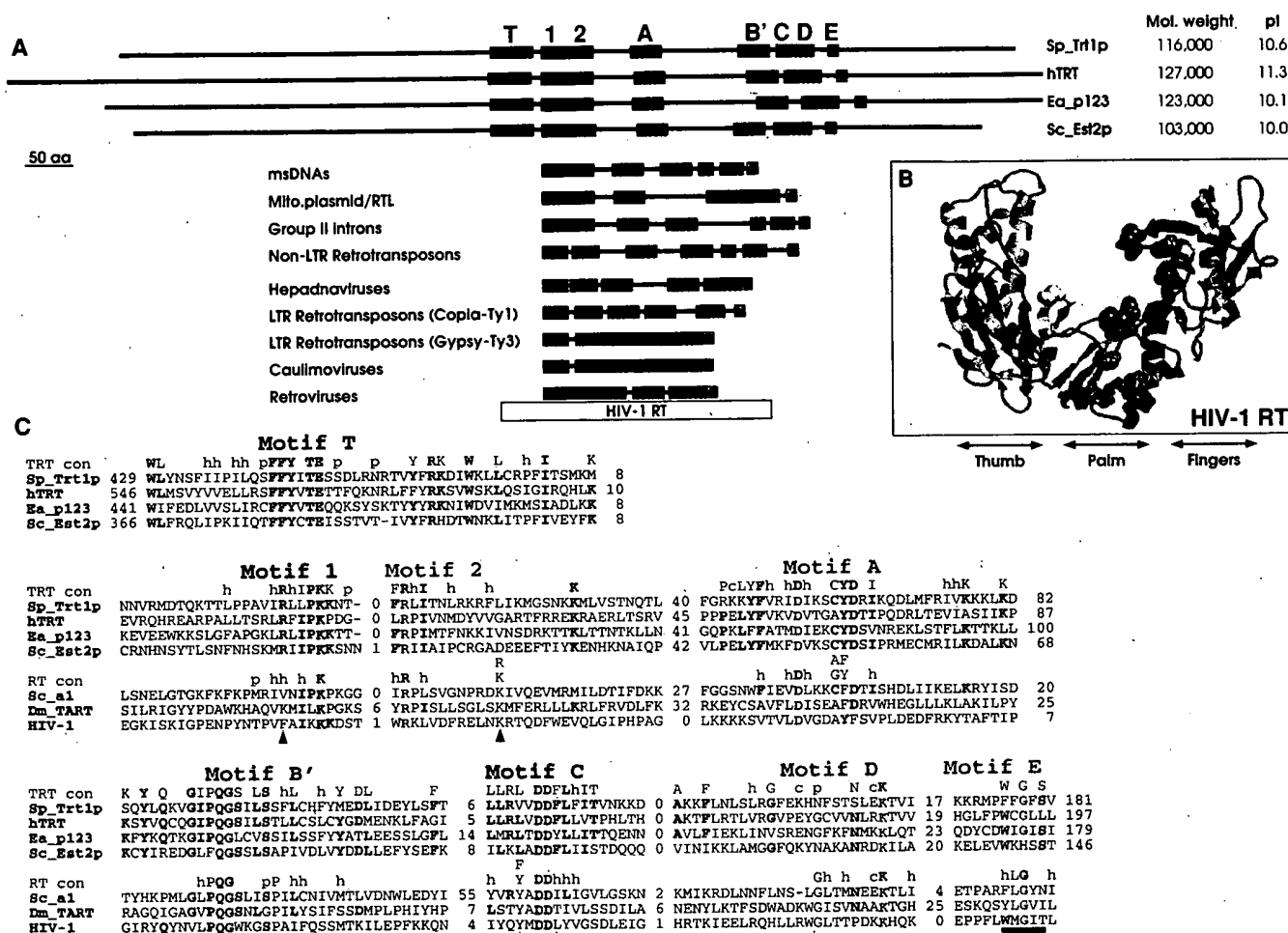
RT-PCR amplification of RNA from 293 cells and from testis each gave two products differing by 182 bp (20). The larger and smaller products from testis RNA were sequenced and found to correspond exactly to pGRN121 and the EST cDNA, respectively.

The relative abundance of hTERT mRNA was assessed in six telomerase-negative mortal cell strains and six telomerase-positive immortal cell lines (21) (Fig. 3). The steady-state level of hTERT mRNA was higher in immortal cell lines with active telomerase (6) than in any of the telomerase-negative cell strains tested. Telomerase activity was more strongly correlated with the abundance of hTERT mRNA than with that of telomerase RNA

(hTR) (7). In contrast, the abundance of mRNA for the human p80 homolog TP1 (9) did not correlate with telomerase activity (Fig. 3). Thus, while our proposal that hTERT is the catalytic subunit of human telomerase is based mainly on protein structural features

**Table 1.** Amino acid sequence identity between telomerase reverse transcriptases. Each value is % identity (% similarity in parentheses) based on RT motifs 1, 2, and A through E in Fig 2C.

	hTERT	SpTert1p	Est2p
Ea p123	26 (49)	28 (45)	24 (46)
Est2p	25 (46)	27 (48)	
SpTert1p	30 (47)		

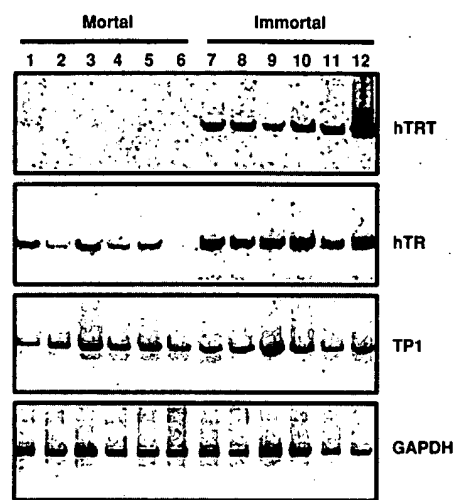


**Fig. 2.** Structure and RT sequence motifs of telomerase proteins. (A) Locations of telomerase-specific motif T and conserved RT motifs 1, 2, and A through E (24) are indicated by colored boxes. The open rectangle labeled HIV-1 (Human Immunodeficiency Virus) RT delineates the portion of this protein shown in (B). *pl*, isoelectric point. (B) The crystal structure of the p66 subunit of HIV-1 RT (Brookhaven code 1HNV). Color-coding of RT motifs matches that in (A). The view is from the back of the right hand, which allows all motifs to be seen. (C) Multiple sequence alignment of telomerase RTs and members of other RT families (Sc\_a1, cytochrome oxidase group II intron 1-encoded protein from *S. cerevisiae* mitochondria; Dm\_TART, reverse transcriptase from *Drosophila melanogaster* TART non-LTR retrotransposable element). Boldface residues indicate identity of at least three telomerase sequences in the alignment. Colored residues are highly con-

served in all RTs and shown as space-filled residues in (B). The number of amino acids between adjacent motifs or to the end of the polypeptide is indicated. TRT con and RT con, consensus sequences for telomerase RTs (this work) and non-telomerase RTs (24) (amino acids are designated h, hydrophobic, A, L, I, V, P, F, W, M; p, polar, G, S, T, Y, C, N, Q; c, charged, D, E, H, K, R). Red arrowheads show some of the systematic differences between telomerase proteins and other RTs. Red rectangle below motif E highlights the primer grip region discussed in the text. Abbreviations for the amino acids are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The nucleotide sequences of the *S. pombe* *trt1+* gene and the human TRT cDNA (pGRN121) have been deposited in GenBank (accession nos. AF015783 and AF015950, respectively).

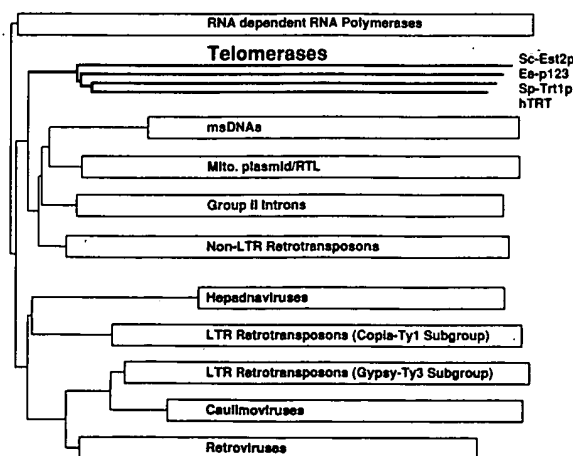
(similarity of RT motifs, the T motif, molecular mass > 100 kD,  $pI$  > 10), the correlation of its mRNA expression level with activity also supports this conclusion.

Sequence alignment of the four telomerase genes revealed features similar to other reverse transcriptases, as well as differences that serve as hallmarks of the telomerase subgroup. The new T motif is one telomerase-specific feature not found in the other RTs examined. Another is the distance between motifs A and B', which is



**Fig. 3.** Expression of hTERT in telomerase-negative mortal cell strains (lanes 1 to 6) and telomerase-positive immortal cell lines (lanes 7 to 12). RT-PCR (21) for hTERT, hTR (human telomerase RNA component), TP1 (telomerase-associated protein related to *Tetrahymena* p80), and GAPDH (to normalize for equal amounts of RNA template) was carried out on RNA from: (1) human fetal lung fibroblasts GFL, (2) human fetal skin fibroblasts GFS, (3) adult prostate stromal fibroblasts 31YO, (4) human fetal knee synovial fibroblasts HSF, (5) neonatal foreskin fibroblasts BJ, (6) human fetal lung fibroblasts IMR90, (7) melanoma LOX IMVI (8) leukemia U251, (9) NCI H23 lung carcinoma, (10) colon adenocarcinoma SW620, (11) breast tumor MCF7, and (12) human 293 cells.

**Fig. 4.** A possible phylogenetic tree of telomerases and retroelements rooted with RNA-dependent RNA polymerases. After sequence alignment of motifs 1, 2, and A through E (178 positions, Fig. 2C) from four TRTs, 67 RTs, and three RNA polymerases, the tree was constructed using the Neighbor Joining method (30). Elements from the same class that are located on the same branch of the tree are simplified as a box. The length of each box corresponds to the most divergent element within that box.



longer in the TRTs than in other RTs (Fig. 2A). These amino acids can be accommodated as an insertion within the "fingers" region of the structure that resembles a right hand (22, 23) (Fig. 2B). Within the motifs, there are a number of substitutions of amino acids (red arrowheads in Fig. 2C) that are highly conserved among the other RTs. For example, in motif C the two aspartic acid residues (DD) that coordinate active site metal ions (22) occur in the context hxDD(F/Y) in the telomerase RTs compared to (F/Y)xDDh in the other RTs (24). Another systematic change characteristic of the telomerase subgroup occurs in motif E, where WxGxSx appears to be the consensus among the telomerase proteins, whereas hLGxxh is characteristic of other RTs (24). This motif E is called the "primer grip" (23), and mutations in this region affect RNA priming but not DNA priming (25). Because telomerase uses a DNA primer, the chromosome 3' end, it is not unexpected that it should differ from other RTs in this region. Given that the simple change from  $Mg^{2+}$  to  $Mn^{2+}$  allows HIV RT to copy a small region of a template in a repetitive manner (26), it is tempting to speculate that some of the distinguishing amino acids in the TRTs may cause telomerase to catalyze repetitive copying of the template sequence of its tightly bound RNA subunit.

Using the seven RT domains (Fig. 2C) defined by Xiong and Eickbush (24), we constructed a phylogenetic tree that includes the four telomerase RTs (Fig. 4). The TRTs appear to be more closely related to RTs associated with msDNA (multicopy single-stranded DNA), group II introns, and non-LTR (Long Terminal Repeat) retrotransposons than to the LTR-retrotransposon and viral RTs. The relationship of the telomerase RTs to the non-LTR branch of retroelements is intriguing, given that the latter elements have replaced telomerase for telomere maintenance in *Drosophila*

(27). However, the most striking finding is that the TRTs form a discrete subgroup, about as closely related to the RNA-dependent RNA polymerases of plus-stranded RNA viruses such as poliovirus as to retroviral RTs. In view of the fact that the four telomerase genes are from evolutionarily distant organisms—protozoan, fungi, and mammals—this separate grouping cannot be explained by lack of phylogenetic diversity in the data set. Instead, this deep branching suggests that the telomerase RTs are an ancient group, perhaps originating with the first eukaryote.

The primary sequence of hTERT and eventual reconstitution of active human telomerase may be used to discover telomerase inhibitors, which in turn will permit additional testing of the anti-tumor effects of telomerase inhibition. The correlation between hTERT mRNA levels and human telomerase activity shown here indicates that hTERT also has promise for cancer diagnosis. With an essential protein component of telomerase now in hand, the stage is set for more detailed investigation of fundamental and applied aspects of this ribonucleoprotein enzyme.

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14. PCR primers were based on motif B' (YARACHAARGGHATYCCCHYARGG) and motif C (NGTNATDARDARTARTCTC), where D = G, A, or T; H = T, A, or C; Y = T or C; R = A or G; and N = G, A, T or C. Approximately  $10^8$  colonies of an *S. pombe* Hind III-cut genomic library in pWH5 [A. Wright, K. Maundrell, W. D. Heyer, D. Beach, P. Nurse, *Plasmid* **15**, 156 (1986)] and  $10^5$  colonies of an *S. pombe* cDNA library [J.-P. Javerzat, G. Cranston, R. C. Allshire, *Nucleic Acids Res.* **24**, 4676 (1996)] were screened.

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27. F F it

15. These cDNA clones terminated in the exon flanked by introns 4 and 5. Poly A<sup>+</sup> RNA from *S. pombe* was reverse transcribed using primer M2-B14 (CCTTG-GAAATCCATTGAAGCCACATGTG). The resulting cDNA was ligated to oligonucleotide pGGGCCGT-GTTGGCCCTAGTTCTCTGCTCddA using T4 RNA li-gase and amplified by two rounds of PCR: in the first round, we used primers M2-B14 and Adapt-Sfi (GAGGAGGAGAAGAGCAGAGAACTAGGCCAACAC-GGCCCC), and in the second, we used primers M2-B15 (AAAGTGGTATGCCAGAAATCTGAAGG-TAAT) and Adapt-Sfi.
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19. A lambda cDNA library from the human 293 cell line, which has high levels of telomerase activity, was partitioned into 25 pools containing ~200,000 plaques each. These were screened by PCR with primers LT5 (CGGAAGAGTGTCTGGAGCAA) and LT6 (GGATGAAGCGGAGTCTGGA) to the 5' region of the #712562 clone insert. Six subpools of one positive primary pool were further screened by PCR. One positive subpool was then screened by plaque hybridization with a probe from the 5' region of clone #712562. One phage was positively identified and the ~4 kbp insert from this clone was excised and subcloned into the pBluescript II SK+ vector (Strat-ogene) as an Eco RI fragment.
20. Polyadenylated RNAs from human testis and from the 293 cell line were amplified using a nested PCR strategy. The first primer set was TCP1.1 (GTGAAG-GCACTGTTCAGCG) and TCP1.15 (CGCGTGGT-GAGGTGAGGTG); the second primer set was TCP1.14 (CTGTGCTGGGCGCTGGACGATA) and bTCP6 (AGCTTGTTCTCCATGTCCGCCGTAG).
21. hTRT mRNA was amplified using oligonucleotide primers LT5 and LT6 (19) for 31 cycles (94°C for 45 s, 60°C for 45 s, 72°C for 90 s). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was amplified using primers K136 (CTCAGACACCAT-GGGGAAGGTGA) and K137 (ATGATCTTGAG-GCTGTGTGTCATA) for 16 cycles (94°C for 45 s, 55°C for 45 s, 72°C for 90 s). hTR was amplified using primers F3b (TCTAACCCCTAACTGAGAAGGGCG-TAG) and R3c (GTTTGCTCTAGAATGAACGGTG-GAAG) for 22 cycles (94°C for 45 s, 55°C for 45 s, 72°C for 90 s). TP1 mRNA was amplified using prim-ers TP1.1 (TCAAGCCAAACCTGAATCTGAG) and TP1.2 (CCCGAGTGAATCTTTCTACGG) for 28 cy-cles (cycles same as for hTRT). Reaction products were resolved on an 8% polyacrylamide gel, stained with SYBR Green I (Molecular Probes, Eugene, OR).
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31. We thank R. Adams, B. Lastelic, L. Tonkin, and F. Wu for expert technical assistance; C. Chapon, J. P. Cooper, R. Gutell, E. Jabri, and J. Sperger for dis-cussions; R. Allshire and J. A. Wise for plasmids and yeast strains; C. Mattison and the L. Pillus lab for help with microscopy; and A. Sirimarco for manuscript preparation. An *S. pombe* cDNA library was provid-ed by C. J. Norbury and B. Edgar. Supported by NIH grant GM28039 (T.R.C.).

23 June 1997; accepted 22 July 1997

## Contrasting Genetic Influence of CCR2 and CCR5 Variants on HIV-1 Infection and Disease Progression

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The critical role of chemokine receptors (CCR5 and CXCR4) in human immunodeficiency virus-type 1 (HIV-1) infection and pathogenesis prompted a search for polymorphisms in other chemokine receptor genes that mediate HIV-1 disease progression. A mutation (CCR2-64I) within the first transmembrane region of the CCR2 chemokine and HIV-1 receptor gene is described that occurred at an allele frequency of 10 to 15 percent among Caucasians and African Americans. Genetic association analysis of five acquired im-munodeficiency syndrome (AIDS) cohorts (3003 patients) revealed that although CCR2-64I exerts no influence on the incidence of HIV-1 infection, HIV-1-infected individuals carrying the CCR2-64I allele progressed to AIDS 2 to 4 years later than individuals homozygous for the common allele. Because CCR2-64I occurs invariably on a CCR5-+-bearing chromosomal haplotype, the independent effects of CCR5-Δ32 (which also delays AIDS onset) and CCR2-64I were determined. An estimated 38 to 45 percent of AIDS patients whose disease progresses rapidly (less than 3 years until onset of AIDS symptoms after HIV-1 exposure) can be attributed to their CCR2-+/+ or CCR5-+/+ genotype, whereas the survival of 28 to 29 percent of long-term survivors, who avoid AIDS for 16 years or more, can be explained by a mutant genotype for CCR2 or CCR5.

The nexus of chemokine immunobiology and AIDS pathogenesis has revealed un-tapped avenues for resolving patterns of HIV-1 disease progression, for clarifying epidemiologic heterogeneity, and for design of therapies (1-6). Identification of the CC-chemokines, RANTES, MIP1α and MIP1β, as suppressor factors produced by CD8 cells that counter infection by certain HIV-1 strain infections (7) previewed the critical identification of two chemokine re-ceptor molecules, CXCR4 (formerly named LESTR/fusin) and CCR5 (formerly CKR5), as cell surface coreceptors with CD4 for HIV-1 infection (8-13). Additional che-mokine receptors CCR2 and CCR3 also

have been implicated as HIV-1 coreceptors on certain cell types (12-14). HIV-1-in-fected patients harbor predominantly mac-rophage-tropic HIV-1 isolates during early stages of infection, but accumulate increas-ing amounts of T cell-tropic strains just before accelerated T cell depletion and pro-gression to AIDS. The identification of "dual"-tropic HIV-1 strains over the course of infection suggests that such strains may represent an intermediate between macro-phage- and T cell-tropic populations (11-13, 15). This tropic transition indicates that viral adaptation from CCR5 to CXCR4 receptor use may be a key step in progres-sion to AIDS (16).

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